DATA EVALUATION RECORD
FISH LIFE-CYCLE TOXICITY TEST
§72-5

1. **CHEMICAL:** Novaluron — PC Code No.: 124002

2. **TEST MATERIAL:** Novaluron Technical — Purity: 100.3%

3. **CITATION:**

Author: Caunter, J.E., and T.D. Williams

Title: Novaluron: Determination of Effects on the Reproduction of Fathead Minnow (*Pimephales promelas*)

Study Completion Date: December 20, 2000

Laboratories: Brixham Environmental Laboratory
Astra Zeneca UK Limited
Brixham Devon, UK

Sponsor: Makhteshim Chemical Works Ltd.
P.O. Box 60
Beer Sheva, Israel

Laboratory Report ID: BL6993/B; AH0411A

MRID No.: 45638214

DP Barcode: D285479

4. **REVIEWED BY:** Rebecca Bryan, Staff Scientist, Dynamac Corporation

Signature: Rebecca Bryan **Date:** 4/1/03

APPROVED BY: Christie E. Padova, B.S., Staff Scientist, Dynamac Corporation

Signature: Christie E. Padova **Date:** 4/1/03

5. **APPROVED BY:** Bill Evans, Biologist, OPP/EFED/ERB - I

Signature: William Evans **Date:** 11/3/03

6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Pimephales promelas*

Age of Test Organism: 11 months old (F_0 generation)

Definitive Test Duration: 96 Days

Study Method: Flow-through

Type of Concentrations: Mean-measured

7. CONCLUSIONS:

The 96-day chronic toxicity of Novaluron Technical to the early- life stage of Fathead Minnow (*Pimephales promelas*) was studied under flow-through conditions. Adult fish (approximately 11 months old) were exposed to the test material at nominal concentrations of 0 (negative and solvent controls), 0.03, 0.3, and 3.0 μg/L. The solvent carrier was dimethylformamide, at 2.35 μL/L. Mean-measured concentrations were <LOQ (<0.05), 0.315, and 2.986 mg a.i./L, respectively, for the test groups. Analyses of the control groups revealed significant contamination in approximately half of the samples analyzed (at up to 0.570 μg/L). The test system was maintained at 24.1 to 25.2°C and pH 7.1-7.8.

Starting on Day 15, the eggs were collected from spawning tiles. The F_0-generation exposure was terminated on exposure Day 47. The F_1-generation exposures were terminated on Day 28 post-hatch. Endpoints assessed included survival of F_0-generation fish, egg production, hatching success, survival of F_1-generation fish (Day 28 post-hatch), and wet weight and length of F_1-generation fish (Day 28 post-hatch).

No treatment-related effects were observed on any endpoint.

This study is classified as INVALID. Novaluron was detected in control water samples throughout the study, and the study authors reported that this may have been due to contamination from airborne particles during the in-life phase of the study (p. 52). Although of the 50 samples analyzed, 22 samples were either <LOD (0.02 μg/L) or <LOQ (<0.05 μg/L), the remainder of samples had Novaluron at concentrations that often overlapped the 0.3 μg/L (nominal) group. Since significant contamination of Novaluron was observed in both of the control groups, data obtained from this study are considered unreliable.

Results Synopsis:

NOEC: Not determined (Invalid study)
LOEC: Not determined (Invalid study)
MATC: Not determined (Invalid study)

Most Sensitive Endpoint: Not determined (Invalid study)

8. ADEQUACY OF THE STUDY:

A. Classification: Invalid

B. Rationale: Significant contamination of Novaluron was observed in both control groups.

C. Repairability: This study may not be upgraded. For fulfillment of the §72-5 guideline requirement, a new study should be submitted.

9. GUIDELINE DEVIATIONS:

1. Novaluron was detected at significant concentrations (≤0.570 μg/L) in both the negative and solvent control dilution water (Tables II through XIV of Appendix 3, pp. 56-68). Only in about half of the control samples analyzed was Novaluron concentrations below the limit of quantitation (0.05 μg/L).

2. The study design did not follow FIFRA Guideline §72-5. In this study, 11-month old Fathead minnow were exposed for up to 47 days, and offspring were maintained for a 28-day exposure period. In a fish life-cycle toxicity test (§72-5), exposure commences with embryos (<24-hours old), and continues through development and ultimately reproduction (40 weeks); second-generation organisms are then exposed for 8 weeks.

3. The time required to hatch was not assessed (for second-generation embryos).

4. The actual number of embryos used for "hatchability" and "early life stage" studies were not reported.

5. De-chlorinated tap water was used as the dilution water.

6. The measured pH range (7.1-7.8) slightly exceeded the recommended range (7.2-7.6).

7. Dissolved oxygen was not provided in terms of percent saturation.

8. Three concentrations were tested instead of the required five test concentrations.

10. SUBMISSION PURPOSE: This study was submitted to provide data on the toxicity of Novaluron to the life cycle of fathead minnows for the purposes of chemical registration.

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species Prefer Sheepshead minnow (*Cyprinodon variegatus*) or Fathead minnow (*Pimephales promelas*).	Fathead minnow (*Pimephales promelas*)
Source and Acclimation	Fish (approximately 5 months old) were obtained from brood stock maintained at Brixham Environmental Laboratory. Fish were held for an additional 6 months prior to study initiation.
Age at beginning of test Embryos, 2 to 24 hours old	Adults, 11 months old

Guideline Criteria	Reported Information
Feeding Fish should be fed at least twice daily and should not be fed for at least 24 hours prior to test termination.	The larvae, juveniles, and adults were fed three times per day on weekdays and twice per day at weekends. The adults were fed frozen adult brine shrimp supplemented with high protein pelleted fish food. The larvae were fed 2 mL of freshwater rotifers (*Brachionus plicatilis*) per replicate tank from hatch day to post-hatch day 7. Starting at post-hatch day 7, the larvae were also fed from 0.67 mL to 3.0 mL of live brine shrimp (*Artemia salina*) per fry. From post-hatch days 16 to 26, the larvae were also fed with high protein pelleted fish food, *ad libitum*, once daily. Fish were not fed at least 24 hours prior to test termination.
Embryo Exposure (4 to 5 Days) Embryos (≤24 hours old) from at least 3 separate spawns should be randomly distributed to embryo cups. A minimum of 50 embryos (≤24 hrs old) per replicate cup, 4 cups per treatment should be used. Parameters measured: · Survival of embryos · Time required to hatch · Hatching success · Survival of fry for 4 weeks Dead and fungused embryos should be counted and removed daily.	Not performed.

Guideline Criteria	Reported Information
Larval-Juvenile Exposure (From Hatch to 8 Weeks) After hatching, each group of larvae is randomly reduced to a minimum of 25 fish and released in replicate larval growth chambers. The random selection must include any fish that are lethargic or deformed. Parameters measured: 9. Fish survival (determined by counting the number of live fish in each replicate growth chamber weekly). 10. Total lengths (mm) of all fish at 4 and 8 weeks after hatching.	Not performed.
Juvenile-Adult Exposure (From 8 weeks posthatch to the end of the spawning phase [32-40 weeks]) At 20-24 weeks after hatching, mature fish are placed in a spawning tank of the same concentration (4 males and 4 females randomly chosen and assigned). The spawning tank is divided into 4 individual spawning chambers with appropriate spawning substrates. The substrates are examined daily and embryos removed, counted, and recorded separately for each pair. For fathead minnow, adult exposure should be terminated when no spawning occurs for one week. For sheepshead minnow, testing should be terminated after spawning is observed for 2 weeks.	Adult Exposure (Days 0-47) On Day 0, four males and six females (11 months old) were impartially assigned to each duplicate test vessel. On Day 14, the fish were paired into one of four breeding chambers per replicate vessel. From Day 15, the spawning substrates were examined daily and embryos removed and counted. Adult exposure was terminated on Day 47. Parameters measured: · Survival of adults · Egg production

Guideline Criteria	Reported Information
Second Generation Embryo Exposure (4 to 5 days) 50 embryos from each conc. level are randomly selected and transferred to incubation cups for hatch. Use the same test procedures as those for parental generation. Embryos not selected are discarded.	Embryo Exposure (two "hatchability" trials from each breeding pair commenced on Day 15) When possible, ≥50 embryos (age not specified; actual number of embryos not reported) from single female spawnings were randomly distributed to embryo cups. There were two replicate vessels per exposure, with two chambers per vessel and one embryo cup per chamber. Parameters measured: · Survival of embryos · Hatching success Dead embryos were counted and removed daily.
Second Generation Larval-Juvenile Exposure (From Hatch to 4-8 weeks) After hatching, 25 larvae are released in each growth chambers (2 chambers per treatment). Each group of 2nd generation fish is terminated 8 weeks after hatching. Fish are blotted, weighed, and measured before being discarded.	Larval-Juvenile Exposure (From Hatch to 4 Weeks) On Day 20, two (if possible) "early life stage " (ELS) studies commenced for each replicate of the controls and treatment groups. The number of larvae used in the studies was not reported. Parameters measured: · Survival and signs of toxicity of fry/juvenile fish · Total lengths (mm) of all surviving fish at 28 Days post-hatch. · Weights (g) of all surviving fish at 28 Days post-hatch.

Comments: The study design did not follow guideline requirements. The test began with adult fish instead of embryos, and second-generation minnows were only maintained for 4 weeks instead of the required 8.

B. Test System

Guideline Criteria	Reported Information
Test Water Sheepshead Minnow 1. Natural seawater (sterilized and filtered) or a commercial mixture. 2. Natural seawater with a salinity of ≥15 parts per thousand (weekly range of salinity <6% and monthly pH range <0.8 pH units). Fathead Minnow 1. Reconstituted water or water from unpolluted well or spring (sterilized and tested for pollutants). 2. Hardness of 40 to 48 mg/L as $CaCO_3$ and pH of 7.2 to 7.6.	N/A 1. De-chlorinated tap water. The water was filtered with activated carbon and mesh filters, and de-chlorinated with sodium thiosulphate, and salts were added. The results of periodic analysis for selected contaminants were provided. 2. Hardness of 40.0-51.7 mg/L as $CaCO_3$ and pH of 7.1-7.8.
Test Temperature Sheepshead: 30°C. Fathead: 25°C and should not remain outside the range of 24 to 26°C for more than 48 hours.	N/A 24.1 to 25.2°C.
Photoperiod 16-hour light/8-hour dark. Light intensity of 10-100 lumens at water surface.	16-hours light/8-hours dark with a 10-minute transition period. Light intensity was 530 to 610 Lux.

Guideline Criteria	Reported Information
Dosing Apparatus 1. Intermittent flow proportional diluters or continuous flow serial diluters. 2. A minimum of 5 toxicant concentrations with a dilution factor $\leq$0.5. 3. One control should be used.	1. Continuous-flow diluter. 2. Three toxicant concentrations with a dilution factor of 0.1. 3. Negative and solvent controls were used.
Toxicant Mixing 1. Mixing chamber recommended but not required. 2. Test solution completely mixed before introduction into the test system (aeration should not be used for mixing). 3. Flow splitting accuracy must be within 10% and periodically checked.	1. A mixing chamber was used for each toxicant level. 2. Yes 3. Mixing and flow splitting chambers were checked twice per week.
Exposure System/Test Vessels Exposure tanks should be all glass or glass with a plastic or stainless steel frame (30.5 x 30.5 x 91.4 cm or 30.5 x 30.5 x 61 cm for fathead, and 45 x 90 x 26 cm for sheepshead). Larval chambers should have glass bottoms and drains that allow water to be drawn down to 3 cm. Test water depth in adult tanks and larval chambers should be a minimum of 15 cm.	Adult exposure tanks were 54 L glass aquaria (610 mm x 305 mm x 310 mm), with a fill volume of 45 L. Progeny exposure tanks were 12 L glass aquaria (305 x 205 x 210 mm), with a fill volume of 9.5 L. It was not specified if larval chambers had drains to allow for water level reduction.

Guideline Criteria	Reported Information
Embryo and Fry Chambers 120 mL glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen. Chambers can be oscillated vertically using rocker arm apparatus (2 rpm motor) or placed in separate chambers with self-starting siphons.	157 mL glass tubing (50-mm diameter, 80 mm length) and 0.47 mm^2 nylon mesh screen bottom. The embryo cups were suspended in the water column of each chamber and oscillated vertically (2 rpm).
Flow Rate Flow rates to adult tanks or larval chambers should provide 90% replacement in 8-12 hours, and maintain DO at above 75% of saturation. The toxicant level cannot drop below 20% with fish in the tank.	The flow-splitting devices supplied approximately 7 tank volumes per day to each of the adult tanks and approximately 12 tank volumes per day to each of the progeny tanks. The DO ranged from 6.2 to 8.4 mg/L during the study (not provided in terms of percent saturation).
Aeration Dilution water should be aerated to insure dissolved oxygen concentrations at or near 100% saturation. Test tanks and embryo chambers should not be aerated.	Test solutions were not aerated during the study.

C. Chemical System

Guideline Criteria	Reported Information
Nominal Concentrations Minimum of 5 concentrations and a control, all replicated; plus solvent control if appropriate. Toxicant conc. must be measured in one tank at each toxicant level every week.	0 (negative and solvent controls), 0.03, 0.3, and 3.0 μg/L. Toxicant concentrations were measured from each test concentration prior to test initiation, days 0, 3, and 7, and weekly thereafter during the test.

Guideline Criteria	Reported Information
Other Variables 1. DO must be measured at each conc. at least once a week. 2. Test water temp. must be recorded continuously. 3. Freshwater: A control and one conc. must be analyzed weekly for pH, alkalinity, hardness, and conductance. Natural seawater: must maintain a constant salinity and not fluctuate more than 6% weekly; monthly pH range <0.8 pH units.	1. DO was measured in each replicate aquaria at test initiation and once weekly during the test. 2. Temperature measured in each replicate aquaria at test initiation and once weekly during the test. Temperature was also continuously monitored in one negative control replicate. 3. pH was measured in each replicate aquaria at test initiation and once weekly during the test. pH, alkalinity, hardness, and conductance were determined 12 times during the study.
Solvents Should not exceed 0.1 mL/L in a flow-through system. Acceptable solvents are: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.	Dimethylformamide (DMF), 2.35 μL/L

Comments: None.

12. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. This study was conducted in compliance with GLP standards set forth by the United Kingdom, EC Council Directive, OECD, U.S. EPA, and Japan Ministry of Agriculture, Forestry and Fisheries.
Data Endpoints must include: · survival of F_0 and F_1 embryos, time required to hatch, and hatching success; · survival and total length of F_0 fish at 4 and 8 weeks after hatching; · weights and lengths of F_1 fish at 8 weeks; · incidence of pathological or histological effects; and · observations of other effects or clinical signs.	Data Endpoints included: · Survival of F_0 fish · Egg production from F_0 fish · Hatching success of F_1 embryos · Survival, total length, and total wet weights of F_1 fish after 4 weeks
Raw data included?	Yes

F_0 Results:

Nominal Conc. (μg/L)	Mean Measured Conc. (μg/L) (SD)	Total No. of Eggs Produced
Negative Control	Not determined[1]	468
Solvent Control	Not determined[1]	486
0.03	<0.05[2]	586
0.3	0.315 ± 0.159	483
3.0	2.986 ± 1.204	750

[1] Significant contamination of the control groups was observed. Only in approximately half of the samples analyzed was Novaluron below the limit of quantitation (0.05 μg/L); in remaining control samples, Novaluron was detected at ≤0.570 μg/L.

[2] Mean-measured concentrations for the 0.03 μg/L (nominal) level were below the limit of quantitation (0.05 μg/L).

Toxicity Observations: No treatment-related effects were observed on survival of the F_0 generation fish (p. 18, and Table 6, p. 27). One male from the highest concentration died on Day 37 and this mortality was deemed insignificant.

No significant treatment-related effects were observed for egg production. The treatment groups were compared to the negative and solvent controls both individually and pooled.

F_1 Results:

Nominal Conc. (µg./L)	Mean Measured Conc. (µg./L) (SD)	% Hatch	28-Day Post-Hatch % Survival		28-Day Post-Hatch Length (mm)		28-Day Post-Hatch Wet Weight (g)	
			ELS1[3]	ELS2	ELS1	ELS2	ELS1	ELS2
Negative Control	Not determined[1]	73	91	88	17.7	19.3	109.4	134.5
Solvent Control	Not determined[1]	85	97	96	18.5	19.8	94.2	121.8
0.03	<0.05[2]	84	77*	82*	18.8	19.3	101.8	113.5
0.3	0.315 ± 0.159	72	93	98	19.3	19.7	106.1	115.5
3.0	2.986 ± 1.204	69	82*	92	17.7	19.8	88.8	125.0

[1] Significant contamination of the control groups was observed. Only in approximately half of the samples analyzed was Novaluron below the limit of quantitation (0.05 µg/L); in remaining control samples, Novaluron was detected at ≤0.570 µg/L.

[2] Mean-measured concentrations for the 0.03 µg/L (nominal) level were below the limit of quantitation (0.05 µg/L).

[3] ELS = Early life stage study

* Significantly reduced compared to the solvent control group.

Toxicity Observations: No significant treatment-related effects were observed for egg hatchability. The treatment groups were compared to the negative and solvent controls both individually and pooled.

The survival of both early life stage (ELS) studies ranged from 77 to 98%. Reductions in survival were significant in the 0.03 (both ELS studies) and 3.0 µg/L (ELS 2 study only) treatment groups, compared to the solvent control group. The study authors reported that since survival was not impaired at the intermediate concentration (0.3 µg/L), findings of reduced survival in the 0.03 µg/L treatment must be treated cautiously, and that it is best to consider the results for survival alongside the results for growth.

There were no significant differences in the lengths of treatment groups compared to the individual and pooled control groups. For wet weight analyses, significant differences were observed between the controls groups, and thus these groups were not pooled for comparison. There were no significant differences in the weights of treatment groups compared to the solvent control group.

B. Reported Statistical Results

Endpoints statistically assessed included egg production, hatching success, survival of progeny (Day 28 post-hatch), and wet weight and length of progeny (Day 28 post-hatch).

Survival data were analyzed using Fisher's Exact Test for 2 x 2 contingency tables to identify treatment groups that showed a statistically-significant difference ($p \leq 0.05$) from the negative and solvent control groups. All remaining endpoints were evaluated for normality and for homogeneity of variance prior to analysis of variance (ANOVA). Wilcoxon's Rank Sum Test was used to evaluate difference between treatment and the controls ($p \leq 0.05$). Nominal concentrations were used for all estimations.

The no observed effect concentration (NOEC) is the highest tested concentration at which a measured biological parameter is not statistically different (at the 95% confidence interval) than the control. The lowest observed effect concentration (LOEC) is the lowest tested concentration at which any measured biological parameter is statistically different from the control and above which all concentrations are significantly different. The maximum acceptable toxicant concentration (MATC) was calculated as the geometric mean of the NOEC and the LOEC.

Biological Endpoint	NOEC (μg/L)	LOEC (μg/L)
F_0 hatching success	Not assessed	Not assessed
F_0 4-week survival	Not assessed	Not assessed
F_0 4-week length	Not assessed	Not assessed
F_0 8-week survival	Not assessed	Not assessed
F_0 8-week length	Not assessed	Not assessed
F_0 8-week weight	Not assessed	Not assessed
F_0 test termination survival	3.0	>3.0
F_0 test termination length (Males)	Not assessed	Not assessed
F_0 test termination length (Females)	Not assessed	Not assessed
F_0 test termination weight (Males)	Not assessed	Not assessed
F_0 test termination weight (Females)	Not assessed	Not assessed
F_0 # of spawns/female	Not assessed	Not assessed
F_0 # of eggs/female	3.0	>3.0
F_1 hatching success	3.0	>3.0
F_1 4-week survival	3.0	>3.0
F_1 4-week length	3.0	>3.0
F_1 4-week weight	3.0	>3.0
F_1 8-week survival	Not assessed	Not assessed
F_1 8-week length	Not assessed	Not assessed
F_1 8-week weight	Not assessed	Not assessed

NOEC: 3.0 μg/L **LOEC:** >3.0 μg/L **MATC:** >3.0 μg/L

13. REVIEWER'S STATISTICAL RESULTS:

No endpoints were significantly affected by treatment with Novaluron technical. The NOEC for length (ELS 1 and 2) and egg production were determined visually. The NOEC for percent survival (F_1) was determined using ANOVA (ELS1) or a two-tailed t-test (ELS2). The NOEC for weight (ELS 1 and 2) was determined using ANOVA followed by William's test. Percent hatch data were not normally distributed, so the non-parametric Kruskal Wallis test was used to determine that there were no differences between the treatment groups and the pooled controls. The NOEC and LOEC in this study were 2.986 and >2.986 μg/L, respectively. Determinations of NOEC and LOEC values were conducted using TOXSTAT statistical software. Mean-measured concentrations were used for all estimations.

14. REVIEWER'S COMMENTS:

This study is classified as INVALID. Novaluron was detected in control water samples throughout the study, and the study authors reported that this may have been due to contamination from airborne particles during the in-life phase of the study (p. 52). Although of the 50 samples analyzed, 22 samples were either <LOD (0.02 μg/L) or <LOQ (<0.05 μg/L), the remainder of samples had Novaluron at concentrations that often overlapped the 0.3 μg/L (nominal) group. Since significant contamination of Novaluron was observed in both of the control groups, data obtained from this study are considered unreliable.

The test did not follow the US EPA test guideline for Fish Life-Cycle Toxicity Test (§72-5). In this study, 11-month old Fathead minnow were exposed for up to 47 days, and offspring were maintained for a 28-day exposure period. In a fish life-cycle toxicity test (§72-5), exposure commences with embryos (<24-hours old), and continues through development and ultimately reproduction (40 weeks); second-generation organisms are then exposed for 8 weeks.

The mean-measured concentration for 3.0 μg/L (nominal) was reported as 2.986 μg/L in Appendix 3 (p. 48) and 2.852 μg/L in the Results section (p. 16).

It was not specified if larval chambers had drains to allow for water level reduction.

15. REFERENCES:

Comb, A.L. 1997. Rimon (Pure): Determination of the physico-chemical properties.

Brownlee, K.A. 1965. Fisher's Exact Test For 2 X 2 Tables. Statistical Theory and Methodology in Science and Engineering. 2nd Edition. John Wiley and Sons, 163-166.

D'Agostine, R.B. 1971. An Omnibus Test of Normality for Moderate and Large Size Samples. Biometrika 58, 342-348.

Shapiro, S.S. and Wilk, M.B. 1965. An Analysis of Variance Test for Normality (complete samples). Biometrika 52, 591-611.

Snedecor, G.W. and Cochran, W.G. 1980. Statistical Methods, 252-253.

Hollander, M. and Wolfe, D.A. 1973. Non-parametric Statistical Methods. J. Wiley & Sons Inc.

16. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

ELS1 survival
File: 82141s Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	3	484.600	161.533	3.576
Within (Error)	6	271.000	45.167	
Total	9	755.600		

Critical F value = 4.76 (0.05,3,6)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

ELS1 survival
File: 82141s Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	93.500	93.500		
2	0.03	77.000	77.000	2.835	*
3	0.315	93.000	93.000	0.086	
4	2.852	82.000	82.000	1.976	

Bonferroni T table value = 2.75 (1 Tailed Value, P=0.05, df=6,3)

ELS1 survival
File: 82141s Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	4			
2	0.03	2	16.006	17.1	16.500
3	0.315	2	16.006	17.1	0.500
4	2.852	2	16.006	17.1	11.500

ELS1 survival
File: 82141s Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	93.500	93.500	93.500
2	0.03	2	77.000	77.000	85.000
3	0.315	2	93.000	93.000	85.000

4	2.852	2	82.000	82.000	82.000

ELS1 survival
File: 82141s Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	93.500				
0.03	85.000	1.460		1.94	k= 1, v= 6
0.315	85.000	1.460		2.06	k= 2, v= 6
2.852	82.000	1.976		2.10	k= 3, v= 6

s = 6.721
Note: df used for table values are approximate when v > 20.

ELS2 Survival
Standard Two-Sample t-Test

data: neg control: V1 in DS1 , and solvent control: V2 in DS1
t = -2.4286, df = 2, p-value = 0.1358
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
-23.559285 6.559285
sample estimates:
mean of neg control: 87.5
mean of solvent control: 96

Standard Two-Sample t-Test

data: pooled control: V1 in DS1 , and 0.03: V3 in DS1
t = 1.1479, df = 4, p-value = 0.315
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
-13.83191 33.33191
sample estimates:
mean of pooled control: 91.75
mean of 0.03: 82

ELS1 total weight
File: 82141w Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	3	334.142	111.381	0.651
Within (Error)	6	1026.622	171.104	
Total	9	1360.764		

Critical F value = 4.76 (0.05,3,6)

Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

ELS1 total weight
File: 82141w Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	102.075	102.075		
2	0.03	101.050	101.050	0.090	
3	0.315	106.650	106.650	-0.404	
4	2.852	89.350	89.350	1.123	

Bonferroni T table value = 2.75 (1 Tailed Value, P=0.05, df=6,3)

ELS1 total weight
File: 82141w Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	4			
2	0.03	2	31.153	30.5	1.025
3	0.315	2	31.153	30.5	-4.575
4	2.852	2	31.153	30.5	12.725

ELS1 total weight
File: 82141w Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	102.075	102.075	102.963
2	0.03	2	101.050	101.050	102.963
3	0.315	2	106.650	106.650	102.963
4	2.852	2	89.350	89.350	89.350

ELS1 total weight
File: 82141w Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	102.963				
0.03	102.963	0.078		1.94	k= 1, v= 6
0.315	102.963	0.078		2.06	k= 2, v= 6
2.852	89.350	1.123		2.10	k= 3, v= 6

s = 13.081
Note: df used for table values are approximate when v > 20.

ELS2 weight
File: 82142w Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	2	134.667	67.334	0.156
Within (Error)	5	2158.912	431.782	
Total	7	2293.580		

Critical F value = 5.79 (0.05,2,5)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

ELS2 weight
File: 82142w Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	123.675	123.675		
2	0.03	113.650	113.650	0.557	
3	2.852	121.000	121.000	0.149	

Bonferroni T table value = 2.57 (1 Tailed Value, P=0.05, df=5,2)

ELS2 weight
File: 82142w Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	4			
2	0.03	2	46.266	37.4	10.025
3	2.852	2	46.266	37.4	2.675

ELS2 weight
File: 82142w Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	123.675	123.675	123.675
2	0.03	2	113.650	113.650	117.325

3	2.852	2	121.000	121.000	117.325

ELS2 weight
File: 82142w Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	123.675				
0.03	117.325	0.353		2.02	k= 1, v= 5
2.852	117.325	0.353		2.14	k= 2, v= 5

s = 20.779
Note: df used for table values are approximate when v > 20.

% hatch
File: 8214h Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	solvent control	84.867	84.867	620.500
2	neg control	72.667	72.667	398.500
3	0.03	83.538	83.538	493.000
4	0.315	71.667	71.667	356.500
5	2.852	69.250	69.250	477.500

Calculated H Value = 3.592 Critical H Value Table = 9.490
Since Calc H < Crit H **FAIL TO REJECT Ho:All groups are equal.**

% hatch
File: 8214h Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 5 4 2 3 1
5	2.852	69.250	69.250	\
4	0.315	71.667	71.667	. \
2	neg control	72.667	72.667	. . \
3	0.03	83.538	83.538	. . . \
1	solvent control	84.867	84.867	. . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,5) = 2.807 Unequal reps - multiple SE values